

ever, the formation of **3** from **1** does not require such a scheme, since nascent "W(NAr)₂(NHAr)Cl" could be deprotonated *intermolecularly* and since the reaction of halide ion with neutral "W(NAr)₃L" (formed by any reaction sequence) appears facile.

These experiments underscore the use of highly basic amido ligands in a sacrificial sense to effect sequential α hydrogen abstractions, a task that is often consigned to carbanion equivalents.^{8,19} Of particular interest will be the reactivity of the W(=NR)₃ functional group if 16-electron, presumably trigonal-planar W(NAr)₃ can be prepared.

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Supplementary Material Available: Analytical and spectroscopic data for compounds **1-6** and tables of crystal data, data collection parameters, atomic positional and thermal parameters, bond distances, and bond angles for [Li(THF)₄][W(NAr)₃Cl] (**3**) (Ar = 2,6-diisopropylphenyl) (8 pages); listing of observed and calculated structure factors for **3** (9 pages). Ordering information is given on any current masthead page.

(19) See, for example: Mayer, J. M.; Curtis, C. J.; Bercaw, J. E. *J. Am. Chem. Soc.* **1983**, *105*, 2651.

Selective Acylation of Peptides Catalyzed by Lipases in Organic Solvents

Lucia Gardossi,[†] Daniele Bianchi,[‡] and Alexander M. Klibanov*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

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The covalent attachment of carboxylic acids is one of the most ubiquitous and important posttranslational modifications of peptides *in vivo*.¹ In order to better understand the function and biochemical significance of such common acylations¹ as acetylation, myristoylation, and palmitoylation, the *in vitro* synthesis of selectively acylated peptides, with the possibility of varying the modification sites, should be very helpful. In addition, peptides acylated with fatty acids become capable of being anchored to liposomes, translocating across lipid membranes, penetrating intact cells, and penetrating through the blood-brain barrier.² However, selective acylation is a formidable task to a chemist due to the presence of numerous reactive groups in peptides and the complexity of the enzymatic systems involved.¹

We report herein a new approach to this problem which is based on our finding³ that lipases, when acting in organic solvents, can catalyze amide-bond formation. The selectivity of lipases in the aminolysis of esters in anhydrous media has been profitably used for asymmetric transformations⁴ and peptide synthesis.⁵ It is now

[†] On leave from the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Trieste, Trieste, Italy.

[‡] Permanent address: Guido Donegani Institute, Novara, Italy.

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applied to selective acylation of peptides.

We prepared⁶ as the initial target molecule, the dipeptide L-Phe- α -L-Lys-O-*t*-Bu (**1**). It has two primary amino groups, the α -NH₂ group of Phe and the ϵ -NH₂ group of Lys, and thus offers a challenge to selective acylation. This dipeptide (5 μ mol) and the activated ester trifluoroethyl acetate⁷ (50 μ mol) were dissolved in 1 mL of anhydrous acetonitrile,⁸ and then 50 mg of one of 15 commercially available lipases⁹ was added to each reaction mixture, followed by vigorous shaking at 45 °C; the reaction progress was monitored by HPLC. After 24 h, in 12 out of 15 reaction mixtures an appreciable disappearance of **1** was observed; in five, the conversion exceeded 50%, and in three, **1**'s HPLC peak completely vanished and a new peak appeared. With the three lipases affording the complete conversion (those from *Pseudomonas* sp., *Aspergillus niger*, and *Chromobacterium viscosum*), the reactions were scaled up 10-fold, and the products were purified by silica gel chromatography (MeOH/CHCl₃, 1:9, as the eluent) and identified by ¹H NMR. All three enzymatic reactions were found¹⁰ to result in a single product, *N*- ϵ -monoacetyl-**1**. Thus all three lipases are highly efficient and regioselective catalysts of acetylation of **1**. In contrast, when this dipeptide was subjected to chemical acetylation (a slight molar excess of acetic anhydride under the same conditions), the product mixture consisted of¹⁰ 73% of *N*- ϵ -monoacetyl-**1**, 4% of *N*- α -monoacetyl-**1**, and 23% of *N,N*- α,ϵ -diacetyl-**1**. The lipases' ϵ -regioselectivity is particularly impressive considering that the enzymatic acetylation of the α -NH₂ group did not occur even though a large excess of trifluoroethyl acetate was still present at the end of the reaction.

Pseudomonas sp. lipase,¹¹ which afforded complete ϵ -monoacetylation of **1** even after a 2-h reaction, was selected for further experimentation. It was established that acetonitrile was not a unique medium for the lipase-catalyzed acetylation: after 24 h the enzymatic reaction was also complete in *tert*-amyl alcohol, tetrahydrofuran, and dichloromethane; significantly, the same exquisite regioselectivity was retained in all the solvents.

The foregoing lipase-catalyzed peptide modification was successfully applied to acyl moieties other than acetyl: under the same experimental conditions as those employed for the acety-

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(6) This dipeptide (and others used in this study) was synthesized via the classical DCC coupling (Sheehan, J. C.; Hess, G. P. *J. Am. Chem. Soc.* **1955**, *77*, 1067) of *N*-CBZ-L-Phe and *N*- ϵ -CBZ-L-Lys-O-*t*-Bu (prepared as described by Roeske, R. *J. Org. Chem.* **1963**, *28*, 1251), followed by deprotection via catalytic hydrogenation. All structures were confirmed by 250-MHz ¹H NMR.

(7) All 2,2,2-trifluoroethyl esters were synthesized as described previously (Riva, S.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 3291).

(8) All the solvents used as reaction media were extensively dried by shaking with 3-Å molecular sieves (which brings the water content below 0.01%) in order to avoid hydrolysis of activated esters.

(9) Lipases from porcine pancreas, *Candida cylindracea*, *Candida lipolytica*, *Pseudomonas* sp., *Aspergillus niger*, *Chromobacterium viscosum*, wheat germ, *Rhizopus arrhizus*, *Rhizopus delemar*, *Rhizopus japonicus*, *Geotrichum candidum*, *Humicola lanuginosa*, *Mucor meheii*, *Mucor javanicus*, and *Penicillium cyclopium*.

(10) All structure elucidations were accomplished by 250-MHz ¹H NMR. Formation of the amide bond through the ϵ -NH₂ group of **1** resulted in the downfield shift of the ϵ -protons from 2.64 to 3.15 ppm (and no effect on the Phe's α -proton). In contrast, the spectrum of *N*- α -monoacetyl-**1** (independently synthesized^{5c} by us) showed a downfield shift of the α -proton from 3.61 to 4.31 ppm (and no effect on the Lys's ϵ -protons). Note that both isomers of monoacetylated **1** and *N,N*- α,ϵ -diacetyl-**1** (prepared by exhaustive chemical acetylation) were readily distinguishable by HPLC, thereby providing a simple routine selectivity-monitoring technique. With the Ala-Lys and Phe-Ser dipeptides, the analysis was similar. In the latter case, acetylation of the Ser's OH group resulted in the downfield shift of the β -protons from 3.86 to 4.87 ppm and a split of the signal of the Ser's α -proton due to its coupling with the β -protons.

(11) Lipoprotein lipase (LPL, hereafter referred to as "lipase") obtained from Amano International Enzyme Co. (Troy, VA) and dried under vacuum prior to use.

lation, **1** was fully acylated after a 24-h incubation with trifluoroethyl esters of both longer aliphatic (octanoic, myristic, and palmitic) and aromatic (phenylacetic) acids. Analysis of the products¹⁰ revealed that in all cases only *N*- ϵ -monoacyl-**1** compounds were formed.

In order to explain the striking ϵ -specificity of lipase with **1**, we examined the acetylation of several other peptides in acetonitrile. It was found that L-Ala- α -L-Lys-O-*t*-Bu had essentially the same reactivity as **1** and the product of the enzymatic reaction was the *N*- ϵ -monoacetyl dipeptide. Hence the phenyl ring in **1** is not responsible for the low reactivity of the α -NH₂ group compared to ϵ . However, the reactivity of the α -NH₂ group in L-Phe-NH₂ was (i) just 3 times lower than that of the ϵ -NH₂ group in **1**, but (ii) 180 times greater than that of the α -NH₂ group in L-Phe-O-*t*-Bu. These data suggest that the lipase is intolerant of a bulky main (but not side) chain of the peptide. This factor, however, plays no role in the reactivity of the ϵ -NH₂ group (presumably due to its remoteness from the main chain), for the rates of enzymatic acetylation of **1** and of the smaller *N*- α -acetyl-L-Lys-NHCH₃ were identical.

Lipase was also found to selectively esterify Ser in a peptide. In fact, the acylation of Ser in the model peptide L-Phe-L-Ser-NH- β -Naph (**2**) was even faster than that of Lys in **1**: the enzymatic conversions in *tert*-amyl alcohol (the former peptide is insoluble in acetonitrile) after 1.5 h were 98% and 52%, respectively. The NMR analysis¹⁰ of the product revealed it to be exclusively *O*-monoacetyl-**2**, thus pointing to lipase's overwhelming preference for Ser's OH vs (chemically more reactive) Phe's NH₂ group.¹² The same result was obtained in the preparative enzymatic palmitoylation of the dipeptide.¹² In contrast, chemical acetylation with equimolar acetic anhydride yielded approximately 50% of the *N,O*-diacetyl-**2**, with the rest being the unreacted dipeptide.

In closing, we have developed a facile methodology for regio- and chemoselective enzymatic incorporation of various acyl moieties into short peptides. We are currently exploring its extension to longer peptides and to proteins.

(12) Thus allowing for the direct acylation of a hydroxyl group without protecting an amino group first.

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Solid-State Photochemical Generation of A Very Stable Phenoxy-Phenoxy Radical Pair

David A. Modarelli and Paul M. Lahti*

Lederle Graduate Research Tower
Department of Chemistry, University of Massachusetts
Amherst, Massachusetts 01003

Clifford George

Code 6030, Naval Research Laboratory
Washington, D.C. 20375

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We have recently reported our finding that aryl oxalate derivatives are convenient, effective unimolecular photochemical sources of aryloxy radicals.¹⁻³ As a result, it is now possible to

explore aspects of aryloxy radical chemistry that were not easily probed by the standard solution bimolecular methods for generating such radicals.⁴ In this paper we report the generation, electron spin resonance (ESR) observation, and thermal stability of remarkably persistent phenoxy-phenoxyl π -radical pairs generated by polycrystalline solid-state photolysis of bis(2,6-di-*tert*-butyl-4-methoxyphenyl) oxalate.

Generation and direct cryogenic observation of radical pairs trapped in proximity in the solid state is a well-established phenomenon.⁵ However, radical pairs typically recombine or react upon warming, and are not readily kept at room temperature. In a case closely related to our work, McRae and Symons⁶ found that 77 K solid-state γ radiolysis (but not UV-vis photolysis) of diaryl carbonates produced both isolated and triplet-paired phenoxy radicals, which disappeared on warming.

We found upon quartz-filtered xenon-arc UV-vis photolysis⁷ of a powder sample of bis(2,6-di-*tert*-butyl-4-methoxyphenyl) oxalate **1** at 77 K under vacuum for ca. 3 min, the production of a reddish sample having a strong central ESR peak⁸ with $g = 2.0051$, attributable to isolated 2,6-di-*tert*-butyl-4-methoxyphenyl radical (2,6-Bu-4-OMe-Phen). In addition, we were able clearly to observe six peaks consistent with the pattern expected for a randomly oriented triplet sample having zero-field-splitting (zfs) parameters $|D'| = 116$ G, $|E'| = 6.0$ G, with $g_{xx} = 2.0060$, $g_{yy} = 2.0057$, and $g_{zz} = 2.0040$. The presence of a $\Delta M_s = 2$ transition in the $g = 4$ region confirms the presence of a triplet state species, which we attribute to interaction of a geminate pair of 2,6-Bu-4-OMe-Phen radicals, constrained in the crystal matrix of the precursor diaryl oxalate (DAO) after double decarbonylation (Scheme I).

The line shape of the triplet ESR spectrum was simulated by the method of Kottis and Levebvre^{9,10} based upon the above values and is shown as curve b of Figure 1. The reddish color and g values are consistent¹¹ with generation of 2,6-Bu-4-OMe-Phen radicals. After the sample was annealed to room temperature and recooled, spectrum a (Figure 1) changed and a new radical-pair spectrum became evident. An example of the new spectrum obtained without a contaminating component of spectrum a is shown in Figure 1 as spectrum c, characterized by zfs parameters $|D'| = 133$ G, $|E'| = 6.7$ G, with $g_{xx} = 2.0060$, $g_{yy} = 2.0056$, and $g_{zz} = 2.0044$ (see simulated curve d). We attribute spectrum a to an initially formed geminate radical pair after photolysis that upon annealing reorganizes to a more stable geometric arrangement in the crystal to give spectrum c, which remains stable for days at room temperature under vacuum. The

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(6) McRae, J. A.; Symons, M. C. R. *J. Chem. Soc. B* **1968**, 428. The zfs values observed for this phenoxy-phenoxyl pair were $|D'| = 144$ G, $|E'| \approx 0$ G.

(7) On the basis of a typical maximum extinction coefficient of $\epsilon \approx 2000$ M⁻¹ cm⁻¹ in these DAO's at ca. 270 nm, for the crystal density of 1.066 g/cm³ for DAO **2**, the irradiating light should penetrate to a distance of ca. 25 μ m; hence, unfiltered photolysis mostly occurs on the surfaces of these solid samples.

(8) All g values in this article were determined relative to solid external diphenylpicrylhydrazyl radical standard with $g = 2.0037$. (Wertz, J. E.; Bolton, J. R. *Electron Spin Resonance: Elementary Theory and Practical Applications*; Chapman and Hall: New York, 1986; p 238 ff.)

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